

CLAIMS

WE CLAIM:

1. A polynucleotide comprising distinct first and second transposase-interacting inverted repeat sequence pairs, each sequence pair having a specificity for binding to and interacting with a distinct transposase enzyme, members of the first sequence pair flanking members of the second sequence pair.
2. A polynucleotide as claimed in Claim 1 wherein the first or second transposase-interacting inverted repeat sequence pair comprises Tn5 wild-type inside end sequences.
3. A polynucleotide as claimed in Claim 1 wherein the first or second transposase-interacting inverted repeat sequence pair comprises Tn5 mosaic end sequences.
4. A polynucleotide as claimed in Claim 1 wherein the first or second transposase-interacting inverted repeat sequence pair comprises Tn5 wild-type outside end sequences.
5. A polynucleotide as claimed in Claim 1 further comprising between the members of the second inverted repeat sequence pair a first sequence for conferring selectability upon a host cell.
6. A polynucleotide as claimed in Claim 5 further comprising, between the members of the second inverted repeat sequence pair, a polynucleotide that encodes a transposase that specifically binds to and interacts with the second sequence pair and still further comprising, between a first adjacent pair of distinct inverted repeat sequences, a second sequence for conferring selectability upon a host cell.

7. A polynucleotide as claimed in Claim 6 further comprising an origin of replication between the first adjacent pair of distinct inverted repeat sequences.

8. A polynucleotide as claimed in Claim 6 further comprising a preselected polynucleotide sequence insert between a second adjacent pair of distinct inverted repeat sequences.

9. A method for producing a gene fusion library, the method comprising the steps of: mixing together, under conditions suitable for *in vitro* transposition, (1) copies of a transposable polynucleotide that comprises distinct first and second transposase-interacting inverted repeat sequence pairs, members of the first sequence pair flanking members of the second sequence pair, the members of the second sequence pair flanking a first sequence for conferring selectability upon a host cell, (2) copies of a first target nucleic acid molecule that comprises a first polypeptide-encoding gene sequence, a first origin of replication and a second sequence for conferring selectability upon a host cell, and (3) a first transposase that binds to and interacts with the first sequence pair, to produce first transposition products; introducing the first transposition products into host cells; selecting host cells that comprise first transposition products wherein the first polypeptide-encoding gene sequence is disrupted by the transposable polynucleotide; mixing (1) the transposition products from the selected host cells and (2) a second target nucleic acid molecule that comprises a second polypeptide-encoding gene sequence, a second origin of replication and a third sequence for conferring selectability upon a host cell, and (3) a second transposase that binds to and interacts with the second sequence pair, to produce second transposition products; introducing the second transposition products into host cells; selecting host cells that comprise second transposition products comprising fusions between the first and the second polypeptide-encoding gene sequences.

10. A method as claimed in Claim 9 wherein the first origin of replication is a conditional origin of replication.

11. A method as claimed in Claim 9 wherein the second origin of replication is a conditional origin of replication.

✓12. A method for deleting a portion of a chromosome of a host cell, the method comprising the steps of:

introducing into the host cell a synaptic complex comprising (1) a first transposase and (2) a transposable polynucleotide that comprises distinct first and second transposase-interacting inverted repeat sequence pairs, members of the first sequence pair flanking members of the second sequence pair, further comprising between the members of the second inverted repeat sequence pair a first sequence for conferring selectability upon a host cell and a polynucleotide that encodes a second transposase that specifically binds to and interacts with the second sequence pair and still further comprising, between a first adjacent pair of distinct inverted repeat sequences, a second sequence for conferring selectability upon a host cell;

selecting host cells in which the transposable polynucleotide has integrated into the chromosome;

inducing expression of the second transposase in the selected host cells; and screening to isolate cells in which a portion of the chromosome is deleted.

13. A method for cloning a portion of a chromosome of a host cell, the method comprising the steps of:

introducing into the host cell a synaptic complex comprising (1) a first transposase and (2) a transposable polynucleotide that comprises distinct first and second transposase-interacting inverted repeat sequence pairs, members of the first sequence pair flanking members of the second sequence pair, further comprising between the members of the second inverted repeat sequence pair a first sequence for conferring selectability upon a host cell and a polynucleotide that encodes a second transposase that specifically binds to and interacts with the second sequence pair and still further comprising, between a first adjacent pair of distinct inverted repeat sequences, a second sequence for conferring selectability upon a host cell and an origin of replication;

selecting host cells in which the transposable polynucleotide has integrated into the chromosome;

inducing expression of the second transposase in the selected host cells; and

screening to isolate cells in which a portion of the chromosome is cloned onto a self-replicating nucleic acid molecule that comprises the origin of replication.

14. A method for inserting a preselected polynucleotide sequence insert into a chromosome of a host cell, the method comprising the steps of:

introducing into the host cell a synaptic complex comprising (1) a first transposase and (2) a transposable polynucleotide that comprises distinct first and second transposase-interacting inverted repeat sequence pairs, members of the first sequence pair flanking members of the second sequence pair and comprising between the members of the second inverted repeat sequence pair a first sequence for conferring selectability upon a host cell and a polynucleotide that encodes a second transposase that specifically binds to and interacts with the second sequence pair and further comprising, between a first adjacent pair of distinct inverted repeat sequences, a second sequence for conferring selectability upon a host cell and further comprising, between a second adjacent pair of distinct inverted repeat sequences, the preselected polynucleotide sequence insert;

selecting host cells in which the transposable polynucleotide has integrated into the chromosome;

inducing expression of the second transposase in the selected host cells; and
screening to isolate cells in which the preselected polynucleotide sequence insert is inserted into the chromosome and the second sequence for conferring selectability upon a host cell is lost from the chromosome.

15. A polynucleotide fusion product between portions of first and second genes that encodes a polypeptide fusion, wherein the portions of the first and second genes are linked by a pair of non-identical transposase-interacting sequences.

16. A chromosome lacking at a site a portion of nucleic acid and comprising at the site a pair of non-identical transposase-interacting sequences.

17. A chromosome containing at a site an inserted portion of nucleic acid flanked by a pair of non-identical transposase-interacting sequences.

18. A self-replicating nucleic acid molecule comprising a pair of non-identical transposase-interacting sequences flanking a portion of a chromosome and an origin of replication.